Investigation note

By-product of the wine industry for control of *Meloidogyne javanica*

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Abstract

Wine industry by-product (WIB) is rich in bioactive compounds, including phenolics. Since root-knot nematodes are suppressed by phenolic compounds, soil amendment with WIB may control these pathogens. We evaluated the effect of wine industry by-product (WIB) on the management of *Meloidogyne javanica* in tomato plants in greenhouse. In the first study, potted soil was amended with 0, 5, 10, 15, 20 25 and 30 g of WIB per kg of soil. Increasing doses of WIB increased plant development. Nematode population was reduced by WIB, especially at the dose of 30 g kg^{-1} of soil. In the second study, a split-root experiment was designed to assess if soil amendment with WIB (30 g kg^{-1} of soil) induces resistance on tomato against *M. javanica*. WIB does not induce tomato resistance against *M. javanica*. This residue controls the nematode by direct contact.

Key words: *Vitis vinifera,* agro-industrial waste, management root-knot nematode.

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Wine industry by-products (WIB) contains high level of nutrients and several bioactive substances, such as phenolic compounds (Makris *et al*., 2007; Reiner *et al*., 2016). Root-knot nematodes are suppressed by phenolic compounds (Aoudia *et al*., 2012). Therefore, soil amendment with WIB can be an additional method for controlling these pathogens. Few researchers have explored the potential of WIB on the management of root-knot nematodes (Nico *et al*., 2004; Pasqua *et al*., 2020).

However, we have noted that studies on dose-response effect of soil amendment with WIB and the potential mechanism of action of this residue against nematodes are still needed to be investigated. To address this knowledge gap, we assessed the effect of soil amendment with doses from 0 to 30 g of WIB per kg of soil on the control of *M. javanica* in tomato plants and we also carried out a split-root experiment to explain how WIB can control nematodes.

WIB was collected in a farm in Mariópolis, Paraná, Brazil, as a fresh fruit residue after pressing grape (*Vitis vinifera* L. varieties ‘White Niagara’ and ‘Pink Niagara’) to produce wine. Next, the residue was dried under the sun until constant weight, ground in a fodder crush (model TRF 650) equipped with a 3 mm sieve, Chemical analysis revealed that WIB had 2.5% of N, 0.52% of P, 1.74% of K, 0.21%of Ca^{2+}, 0.12% of Mg^{2+}, 13 mg kg^{-1} of S, 64 mg kg^{-1} of Zn, 1 005 mg kg^{-1} of Fe, 39 mg kg^{-1} of Mn, 32 mg kg^{-1} of Cu, 36.3 mg $kg^{-1}$of B and C:N ratio of 16.5.

Eight kilograms of autoclaved soil (120 °C for 1 h) were amended with 0, 5, 10, 15, 20, 25 or 30 g kg^{-1} of WIB and put in 30 L plastic bags. After tying, the bag was vigorously shaken manually. The soil was infested with aqueous suspension with 32 000 eggs (first experiment) and 40 000 eggs (second experiment) of *M. javanica*. Soil moisture was adjusted by adding 1.5 L of water into each bag. The bags were kept in the dark at 26 °C for 15 days, when the soil was transferred to 1.1 L plastic pots. Three-week-old tomato *cv* Santa Cruz Kada was transplanted to each pot.

The plants were fertilized and irrigated as required and were grown for 60 days. The experiments were evaluated by measuring plant height, shoot and root biomass, and by counting the numbers of galls and nematode eggs in the roots. The experiments were carried out in a completely randomized design with eight replicates. To assess if WIB can induce resistance in tomato to root-knot nematode, we designed an experiment in which a split-root technique was used (Ferreira, 2012).

Twenty-one-day-old tomato seedlings (10 cm high) were transplanted to 250 ml plastic cups filled with organo-mineral compost (Tecnomax®) and cultivated for 25 days in greenhouse. Upon reaching the height of ~25 cm, plants were pulled out of soil and the roots were eliminated by a transversal cut in the base of the stem.

Next, a longitudinal slit was made with a slender probe about 8 cm from the lower end of the stem and the two parts were inserted each one in a pot containing 500 g of soil amended or not with 30 g of WIB. Nematode was inoculated 10 days later, when roots were formed in both sides of each plant. A suspension with 5 000 eggs was applied into three holes at the base of the stem. Treatments tested are described in (Table 1). Root biomass and the number of galls and eggs were assessed 60 days after soil infestation. The mean maximum and mean minimum were 33 and 13 °C in the first experiment and 34 and 21 °C in the second experiment.

Table 1. Mass of fresh, root number of galls and eggs per split root system of tomato plants developed in soil infested with 5 000 eggs of *Meloidogyne javanica* and amended with wine industry by-product (WIB).

		Treatment	Mass of fresh root (g)	Number of galls	Number of eggs
1	Pot A	Nematodes + soil	2.93 ns	94.67	35 147.23
	Pot B	Soil			
2	Pot A	Soil			
	Pot B	WIB + nematodes + soil	2.12	25*	3 086.11*
3	Pot A	WIB	2.67	158.5	67 825.01
	Pot B	Nematodes + soil			

*= differs from the control treatment (T1- pot A= nematode + soil) by Dunnett's test ($p< 0.05$). ns= not significant by F test ($p> 0.05$).

The data were submitted to normality (Shapiro-Wilk test) and homogeneity of experimental error tests (Levene test) and then were subjected to analysis of variance ($p< 0.05$), processed or not for $\log_{10}(x)$ or $\sqrt{(x)}$. Qualitative data were analyzed by regression analysis and the effect of soil amendment with WIB and the non-amended control in split-root experiment was assessed by Dunnett's test ($p< 0.05$). Increasing doses of WIB increased height and biomass of tomato plants. Soil amendment with WIB at 30 g kg^{-1} of soil increased plant height, shoot and root biomass by 16-63%, 142-246% and 76-104% in the experiments 1 and 2, respectively (Figure 1).

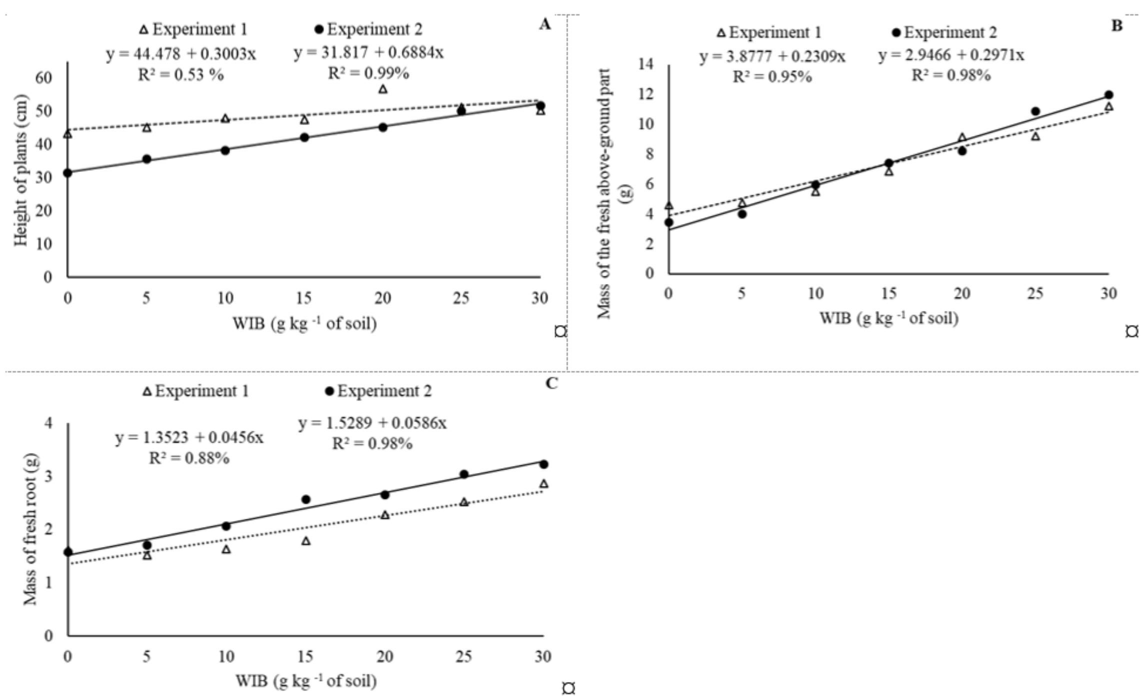

Figure 1. A) height of plants; B) mass of the fresh above-ground part; and C) mass of fresh root of the tomato plants inoculated with 4 000 (experiment 1) and 5 000 eggs of *Meloidogyne javanica* (experiment 2) in soil amended with wine industry by-product (WIB).

WIB reduced the number of galls and eggs of *M. javanica* in tomato plants in both the experiments (Figure 2). In the first experiment, the application of WIB at 30 g kg^{-1} of soil reduced the number of galls per root system and grams galls of root by 76 and 88% (Figure 2A, 2B), whilst the number of eggs per root system and grams of root was reduced by 92-96% (Figure 2C, 2D). In the second experiment, amending the soil with WIB at 30 g kg^{-1} of soil reduced the number of galls per grams of root by 40.5% (Figure B) and eggs on tomato roots from 38 to 72% (Figure 2C, 2D).

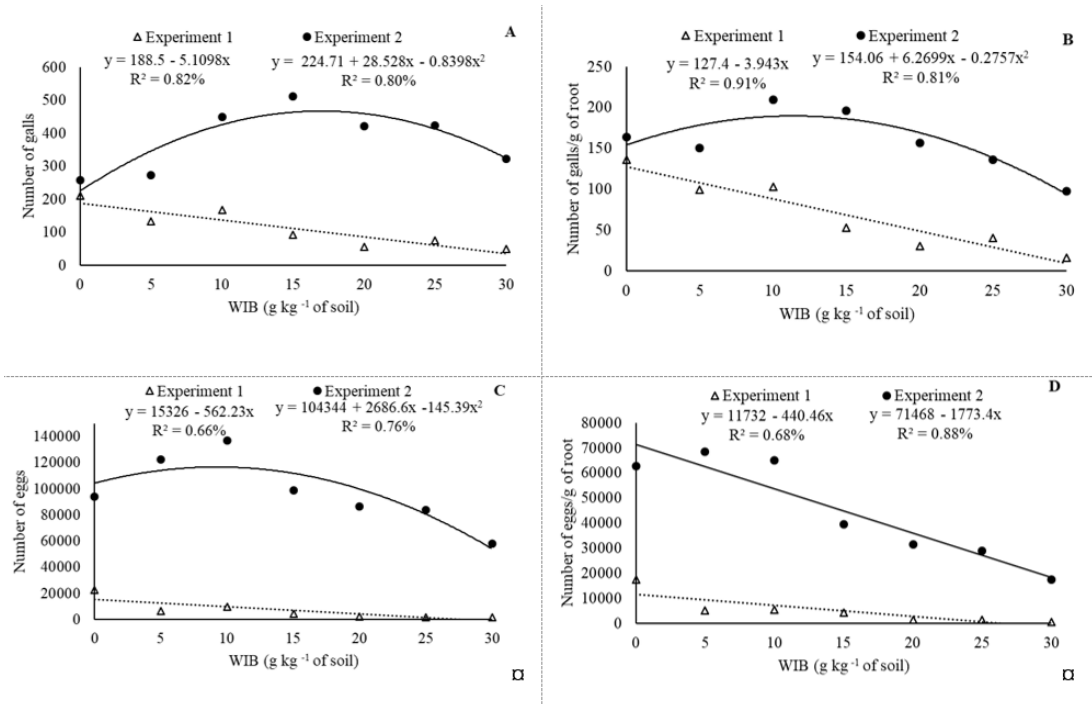

Figure 2. A) number of galls per root system; B) number of galls per gram of root; C) number of eggs per root system; and D) eggs per gram of root, in tomato plants inoculated with 4 000 (experiment 1) and 5 000 eggs of *Meloidogyne javanica*, (experiment 2) in soil amended with wine industry by-product (WIB).

In the split-root system experiment, soil amendment with WIB did not induce resistance in tomato against *M. javanica*, since the nematicidal effect was not observed when WIB was placed in a different pot from nematode eggs (Table 1).

WIB contains a significant quantity of macro-and micronutrients and the release of these elements during decomposition may stimulate plant growth, as we observed in this study. Phosphorus (P), for instance, enhances root growth (Shaheen *et al*., 2007), which will on the uptake of water and other nutrients. Tomatoes should be transplanted at 15 days after soil amendment with WIB to avoid phytotoxicity and favor nutrients bioavailability (Reiner, 2015). Ferreira (2012) reported that tomato growth is higher when the plants are transplanted 14 days after soil amendment with castor bean oilcake.

Soil amendment with nutrient-rich organic materials enhances plant tolerance against nematodes (Rodríguez-Kábana *et al*., 1987). Besides, organic residues with C:N ratios ranging from 14 and 20:1, such as WIB (C/N= 16.5:1) can control plant-parasitic nematodes, due to the release of ammonium and other nitrogen nematicidal compounds (Rodríguez-Kábana *et al*., 1987). WIB is also rich in nematicidal phenolics, including phenolic acids and flavonoids that are released during decomposition (Makris *et al*., 2007; Reiner *et al*., 2016).

In general, the dose of 30 g of WIB per kg of soil was associated with a higher suppression of *M. javanica*, despite some differences between experiment 1 and 2, including inoculum density, greenhouse temperature and behavior of dose-response curves. Probably large amounts of nematicidal substances are released in soil upon the addition of WIB at 30 g kg^{-1}. This dose corresponds to approximately 3% of soil volume or 72 Mg ha^{-1} (soil density of 1.2 g cm^{-3} and 20 cm deep). This quantity can be further reduced if the amendment is incorporated to lesser depths and to treat small areas infested with nematodes (Lopes *et al*., 2008). Large amounts of WIB are generated in the wine industry. Therefore, soil amending with WIB can be an environmentally friendly alternative to manage root-knot nematodes.

Conclusions

Soil amendment with WIB enhances tomato growth and reduces *M. javanica* population, especially at rates of 30 g kg^{-1} of soil. WIB does not induce tomato resistance against *M. javanica*. This residue controls the nematode by direct contact.

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